Microscope Cell Culture Incubator

Product Design Specifications | February 3rd, 2017

Client: Professor John Puccinelli
Advisor: Professor Mitchell Tyler
Team: Jack McGinnity, *Leader*
Jenny Westlund, *BWIG*
Steve Gock, *Communicator*
Trevor Zarecki, *BPAG*
Peter Hartig, *BSAC*

Function: The device should enable the continuous culture of live cells for up to two weeks on an inverted microscope, without impeding imaging capabilities. The cell culture environment must imitate that of an incubator with precise control and readout of temperature, CO_2, and humidity, all within a sterilizable environment.

Client Requirements:

- Temperature control and user interface
- Humidity control and user interface
- CO_2 concentration control and user interface
- Incubation chamber must not impede visibility of cells for imaging
- Accessible for changing cell culture plates and media
- Sterilizable with a standard 70% ethanol solution
- Fit securely on an inverted microscope as to ensure imaging of a consistent location

Design Requirements:

1. **Physical and Operational Characteristics**
    a. ***Cell Culture Related Performance Requirements:*** The device should maintain sterile, incubator-like conditions for 2 weeks. It must maintain the temperature at 37℃ ± 1℃, and re-establish temperature after less than 6 seconds following a 30 second door opening. It must maintain 95-100% humidity within the culture chamber. Finally, it should maintain 5% ±0.5% CO_2 concentration and reestablish concentration after less than 6 seconds following a 30 second door opening.
    b. ***Incubator Housing Related Performance Requirements:*** Incubator housing and any potential condensation must not disrupt optics during imaging. The housing must not limit ability to navigate the full field of the cell culture plate, and not substantially change the distance between the cell culture plate and the objective. Housing materials must be compatible with culture media and be

sterilizable with 70% ethanol solution. The system should also have adequate insulation to prevent internal temperature fluctuations due to external temperature changes.

c. ***Safety:*** Culture environment must be compliant to BioSafety Level 1 standards. All electrical components within the culture environment must be sterilizable and waterproofed, and all circuitry must be rated to the supplied power and current used.

d. ***Accuracy and Reliability:*** For each of the four environmental parameters controlled (temperature, humidity, CO_2 percentage and air sterility), the sensor measurement/readout error and parameter controls must be within the tolerance. The precision of measurements taken during system use are as follows:
   i. Humidity: 95-100% ± 5% of readout value
   ii. CO_2 concentration: 5% ± .5% of readout value
   iii. Temperature: 37℃ ± 1℃ of readout value

   To ensure these environmental parameters are maintained over time, the incubation system will be routinely calibrated against other temperature and CO_2 sensors (about 1x/month).

e. ***Life in Service:*** The incubation chamber should maintain the specified environmental conditions to promote cell life for up to two weeks. The electronic components of the chamber must function under these environmental conditions without recalibration or repair during this time period. When used intermittently, the incubator should function properly for up to a month, after which time recalibrations should be performed.

f. ***Operating Environment:*** The internal portion of the incubation chamber must function in conditions of 95% relative humidity or more, temperatures of 37℃ and CO_2 levels of 5% during operation. If the system is not in use, the incubator will be exposed to normal environmental conditions: room temperature (18-24℃), average indoor relative humidity (30-60%), and atmospheric CO_2 concentration (0.035%). There will be limited dirt exposure inside the incubation chamber, as live cells will be stored in it. Users will be opening and closing the incubation chamber frequently, so the system will also have to adapt to sudden drops in temperature, relative humidity, and CO_2 percentage. It must be possible for the user to perform basic cell culture techniques (such as change cell media) inside the incubation chamber without changing the location of the culture plate. In addition, the surfaces that must be imaged through should maintain their transparency throughout the entire imaging study, without build up of condensation.

g. ***Ergonomics:*** The user will have limited interaction with the incubator itself, except to exchange cell culture dishes in and out of the chamber. The door to the chamber should be easy to open, and allow for enough clearance to fit a cell culture plate, flask, or petri dish inside the incubator.

h. ***Size:*** The interior of the incubation chamber should be at minimum 15.4 cm x 9.4 cm x 2.5 cm tall. The incubation chamber should fit securely on a stage with

dimensions as small as 16.0 cm x 25.0 cm, with a clearance of 5.3 cm tall for the light source.

i. ***Weight:*** Each component of the final product should be no more than 12 kg, such that it is easy to transport between experiments without too much difficulty.
j. ***Materials:*** Materials used for the incubation chamber should not have cytotoxic effects on cells inside their culture dishes, and should be sterilizable with ethanol. The materials should be resistant to corrosion from the high humidity levels. Glass must be used for the bottom surface, and the top surface should not defract light from the light source significantly.
k. ***Aesthetics, Appearance, and Finish:*** The surfaces through which imaging will occur should be transparent, and not result in any aberrations or otherwise compromise the quality of imaging. There should also be a mechanism to protect the experiments from light pollution.

2. Production Characteristics

a. ***Quantity:*** The client needs a total of one microscope cell culture incubator.
b. ***Target Product Cost:*** The target product cost is to be $200, with an understanding that the product would enter the market for around $500 - $1000.

3. Miscellaneous

a. ***Standards and Specifications:*** As the device is currently targeted towards research, the device would not be regulated under any governing standards. Should the device be implemented in the development process by manufacturers, it would fall under ISO 13485 regulation. Further, should the device be used in processes for drug development, FDA Title 21 Chapter I would be the the governing regulatory body.
b. ***Customer:*** The target customers are seeking an affordable, versatile and reliable alternative to the products available in the current on-stage incubation market. The primary customer and client of the project has research focused on microfluidics and biomaterial research, some of the specifications such as size and environmental parameter tolerance reflect the needs of this field.
c. ***Patient-related concerns:*** The product will not have any contact with patients, so patient-related concerns are not applicable.
d. ***Competition:*** There are a variety of systems that have been fabricated for similar purposes, but to the knowledge of the team the device we intend to create would be unique in cost, ease of use, and the ability to be used with a number of microscopes. Stage incubators on the market, such as the Pecon Incubation System 2000 fits all functional requirements of the client but is specifically tailored to fit the Olympus IX71/81 microscope. In contrast, many real-time cell imaging systems exist and are market by large microscope manufacturers such as Nikon[(4)]. The primary deterrent for many potential customers of these devices are the high price and inflexibility of these systems to accommodate a variety of imaging modalities.

References

1. http://eesc.columbia.edu/courses/ees/slides/climate/table_1.html
2. https://www.reference.com/home-garden/normal-room-temperature-2dc5daf4f710aa8
3. http://ehs.uconn.edu/docs/Officecomfort.pdf
4. https://www.nikoninstruments.com/Products/Live-Cell-Screening-Systems/BioStation-CT